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Article

Can Umbilical Cord and Meconium Results Be Directly Compared? Analytical Approach Matters

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Abstract

Maternal drug use during pregnancy is a significant concern. Drug-exposed newborns are often born premature and may suffer from birth defects, neonatal abstinence syndrome and cognitive and developmental delays. Because of this, testing of neonatal specimens is carried out to assess fetal drug exposure during pregnancy. Umbilical cord tissue (UC) and meconium are commonly used specimens for this purpose. However, comprehensive studies comparing drug positivity rates and concentration in the two specimen types are lacking. To this end, 4,036 paired UC and meconium specimens originating from 13 states within the USA were identified, and retrospective analysis of drug positivity rates and drug concentration was performed for 31 analytes in 5 drug classes. Testing for 11-Nor-9-carboxy-tetrahydrocannabinol (THC-COOH) is a separate orderable for UC specimen at our laboratory, so a second data set was created for evaluation of this drug analyte with 2,112 paired UC and meconium specimens originating from 11 states. Testing of UC was performed by semi-quantitative liquid chromatography–tandem mass spectrometry (LC–MS-MS) assays, whereas, for meconium, an immunoassay-based screening preceded LC–MS-MS confirmation tests. Results generated for UC and meconium specimens were therefore compared for a total of 32 drug analytes from 6 drug classes. Drug concentrations for analytes were higher in meconium compared to UC, with the exception of phencyclidine. Despite this, the positivity rates for individual analytes were higher in UC, with the exception of THC-COOH and cocaine. Furthermore, analysis for multidrug positivity revealed that THC-COOH and opioids were the most common multidrug combination detected in both matrices. In conclusion, this study suggests that for most drug compounds, UC was more analytically sensitive to assess neonatal drug exposure by current methodologies. Additionally, by demonstrating that meconium has higher drug concentrations for most compounds, this study sets the stage for developing more sensitive assays in meconium.

Introduction

Prenatal exposure to drugs may come from prescription or illicit/recreational substances (1). These exposures are difficult to quantify and study but can negatively affect neonatal health, contributing to short- and long-term health implications such as premature birth, neonatal abstinence syndrome, birth defects and cognitive and learning disabilities (1–4). Mothers of drug-exposed newborns may face sociolegal consequences when illicit drugs are involved, including accusations of child abuse, which may result in loss of custodial rights (3, 5). Some states in the USA require the providers to report maternal drug use during pregnancy to public health authorities (5). Thus, timely detection of *in utero* drug exposure is critical to neonatal health and the long-term well-being of the child. In addition to maternal history and clinical presentation of the neonate, laboratory testing of umbilical cord tissue (UC) and meconium specimens is used to detect *in utero* drug exposure (3). Currently, meconium is considered the gold standard for neonatal drug testing (6); however, large-scale studies directly comparing drug concentrations and drug detection rates in paired (i.e. originate from the same patient) UC and meconium specimens are limited.

Turnaround times (TATs) for the availability of testing results for each specimen type may also impact the decision to test meconium or UC. Because UC is available at birth and meconium may require several days after birth to pass, testing UC may produce faster TAT than meconium (7). In addition, the specific drug(s) of interest and the number of drug analytes included in the drug detection panels may also have a bearing on specimen choice. At our institution, the UC drug panel consists of 49 analytes as opposed to 36 analytes in the meconium drug panel. Gabapentin, fentanyl and tramadol, for example, are included in the UC panel but not in the current meconium panel. Therefore, the clinical suspicion for drug use, ease of collection, size of the drug panel and hospital logistics may influence which specimen type is tested.

Two previous studies have compared these matrices in terms of concordance and turnaround times and reported somewhat conflicting results. Colby and colleagues demonstrated that drug testing of meconium was more sensitive in assessing neonatal drug exposure, and the concordance between results derived from meconium and UC matrices was about 70% (6, 8). In contrast, Cotten et al. demonstrated that UC was more sensitive compared to meconium and had faster turnaround times (7). These studies were conducted with a relatively small number of specimens collected from single facilities and exhibited different results relative

to which specimen types may be more appropriate for specific analytes. For drug compounds with low positivity rates (e.g. benzodiazepines), it was difficult to determine specimen comparability when the *N* number was small. Patients may also be exposed to multiple drugs *in utero*, the frequency and patterns of which are not well known (9). Lastly, studies comparing the two specimen types are dependent on the specific assay designs and performance characteristics relevant to that study. While testing associated with the Colby study was performed by our laboratories, the details of the methodology have changed substantially since that time, in recognition that the analytical approach is another important variable.

The purpose of this investigation was to directly compare drug testing results obtained with paired meconium and UC specimens originating from multiple centers in the USA to determine their utility in assessing neonatal drug exposure. To this end, two large cohorts of paired specimens ($n = 4{,}036$ and $n = 2{,}112$; Figure 1) were employed to determine and compare positivity rates and concentrations for 32 drug compounds from 6 drug classes in meconium and UC. Common patterns of two-drug and three-drug exposures were also described.

Materials and Methods

Meconium analysis

Drug detection in meconium specimens was carried out using an initial screen [11 separate enzyme-linked immunosorbent assays (ELISAs); Supplementary Table S1 Immunalysis, USA], and specimens positive for one or more assays were reflexed to nine class-based quantitative liquid chromatography–tandem mass spectrometry (LC–MS-MS) assays as previously described (10–12). Briefly, 0.25 g of meconium (aliquot of patient samples, drug-free specimen blank, calibrators and quality control materials) was homogenized in buffer and centrifuged to remove particulate matter. The supernatant was collected for testing. Results for specimens that tested positive by ELISA were confirmed and quantified by LC–MS-MS using class-based assays and several instrument systems. The cut-off concentrations for screening and confirmatory tests in meconium are shown in Supplementary Table S1.

Umbilical cord analysis

Detection of prenatal drug exposure in UC specimens was carried out using semi-quantitative LC–MS-MS assays as previously described (13–16). For all drugs except THC

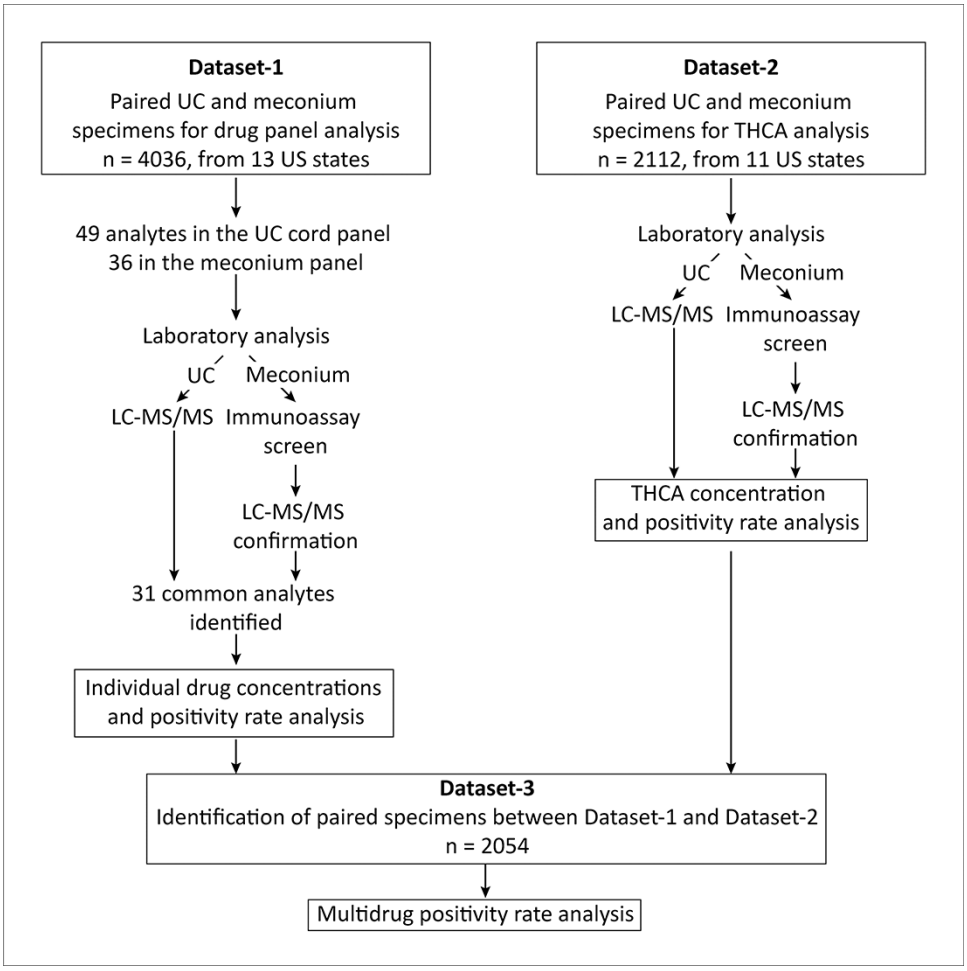

Figure 1. Flow chart depicting specimen selection and study design. Paired UC and meconium specimens were identified for drug panel (Dataset-1) and THC-COOH, Dataset-2 analysis. Subsequently, Dataset-3 was created by identifying common specimens from Dataset-1 and Dataset-2 to perform multidrug pattern analysis. A total of 32 analytes were measured in both specimen types for a side-by-side comparison.

metabolite, 1 g of UC was homogenized in buffer containing β-glucuronidase enzyme (10,000 Fishman units/mL) and isotopically labeled drug standards. Tissue homogenates were incubated at 55°C for 30 min for enzyme hydrolysis, followed by centrifugation. The supernatant was passed through solid-phase cation exchange columns. The resulting eluents were evaporated, and the residues were reconstituted in 10% methanol for analysis on an LC–MS-MS (Agilent 1260 Infinity II, Agilent 6470 Triple Quad) analytical system. Qualitative identity of the analytes was determined by direct comparison of relative retention times, chromatographic quality and comparison of ion mass ratios of analyte mass transitions to that of standards. Semi-quantitative determination of analyte values was made with a single-point calibrator at the cut-off concentration for each analyte. The ratio of quantitative mass transition response of the analyte to that of the internal standard was used to determine the analyte concentration by extrapolation on the calibration curve (forced through the origin). Quality control materials were prepared at 50% and 150% of cut-off concentrations. 11-Nor-9-carboxy-THC (THC-COOH) analysis was performed as described previously (17). Briefly, 1 g of UC tissue was weighed and homogenized in 2.5 mL of methanol and 25 μL of deuterated internal standards solution. Tissue homogenates were centrifuged, and supernatants were treated with 2.5 mL of 0.5 mol/L NaOH solution for 20 min at room temperature on a shaker for alkaline hydrolysis. Subsequently, hydrolyzed supernatants were passed through anion-exchange solid-phase extraction columns (Biotage Evolute AX, USA). Elution was performed using 600 μL of 2% acetic acid in methanol followed by air drying at 40°C for approximately 20 min. The resulting pellets were reconstituted in 200 μL of methanol 40% (v/v) solution and analyzed on an LC–MS-MS analytical system (Agilent 1260 Infinity II, AB Sciex 5500 Triple Quad). The cut-off concentrations for UC are shown in Supplementary Table S1.

Retrospective data analysis

Data generated between 20 May 2019 and 14 November 2020 were retrieved and de-identified at ARUP Laboratories according to the institutional review board approved protocols. Figure 1 describes the approach to study design and data analysis. Specimens targeted for the study originated from 19 medical centers in 13 states. These clients were shown to frequently send UC and meconium specimens from a single birth (paired specimens) for routine clinical testing during the study period. Since UC and meconium are often sent separately, multiple identifiers including client ID, date of birth, sex, medical record number, patient name and zip code were used to verify that the specimen pairs originated from the same birth. Due to the de-identification process, specimens collected from twins or triplets could not be discriminated from singleton births, although it is expected that only approximately 2% of positive specimens would have originated from twins or triplets (18). R-programming was used to align the patient/specimen details. When one of the identifiers did not match, the associated results were not used in the analysis. In the final data set, paired specimens were identified for UC and meconium drug panel testing, wherein 31 common analytes between the two drug panels were analyzed (Dataset-1, $n = 4{,}036$). THC-COOH testing in UC is a separate orderable assay, so it was investigated separately from the large drug panel. In this cohort, paired UC and meconium specimens were identified during the same time frame and for the same clients (Dataset-2, $n = 2{,}112$). For multidrug positivity analysis, Dataset-1 and Dataset-2 were aligned, which resulted in Dataset-3 ($n = 2{,}054$), which was utilized for determining multidrug positivity rates. Quantitative values for the UC assay were extracted from archived raw data files, whereas the quantitative values for the meconium assay were available in the lab information system. Drug concentrations, as well as single and multidrug positivity rates, were analyzed using R-programming, Microsoft Excel and GraphPad Prism programs. Statistical significance between median drug concentrations was calculated using Wilcoxon matched-pairs signed-rank test. Two-tailed z-test was used to calculate statistical significance (i.e. P value) when positivity rates were compared. Alpha was set at 0.05. Figures were prepared using GraphPad Prism (version 8.4.3) and Adobe Illustrator (version 24.3).

Results

Umbilical cord specimens had higher drug positivity rates than meconium, with a few exceptions

First, we compared drug positivity rates in the two specimen types. The total drug positivity rates in Dataset-3 were 56.6% in UC compared to 52.4% in meconium ($P < 0.01$). For meconium, individual drug positivity rates ranged between 0 and 35.0%, while they were between 0.1% and 25.3% for UC (Table I). Interestingly, however, positivity rates for individual analytes varied significantly between the two specimen types.

In the opioid drug class, the positivity rate for meconium ranged between 0.0% and 9.1% compared to 1.0–15.6% in UC. Positivity rates for all individual opioid analytes were higher in UC than in meconium (Table I). In both specimen types, buprenorphine was the most frequently detected opioid with a positivity rate of 15.6% in UC compared to 9.1% in meconium. 6-Acetylmorphine (heroin metabolite) was detected in UC at a rate of 1.0%, whereas no meconium specimens tested positive for this analyte. The oxycodone positivity rate was not significantly different between the two specimen types, but oxymorphone was more frequently detected in UC (4.8%) relative to meconium (1.5%). In contrast to opioids, the THC-COOH positivity rate was higher (35.0%) in meconium compared to UC (25.3%).

In the stimulant drug class, meconium positivity rates were higher than UC (Table I). Amphetamine positivity rate was 6.7% in UC relative to 7.7% in meconium, while the methamphetamine positivity rate in UC was 6.8% in comparison to 7.8% in meconium. In both cases, however, there was no statistically significant difference (Table I). Cocaine positivity was confirmed if any of the four cocaine analytes [i.e. cocaine, benzoylecgonine, m-hydroxybenzoylecgonine (MOH) or cocaethylene] were detected. The meconium positivity rate for cocaine and metabolites was higher (5.6%) than UC (4.0%), corresponding to the high prevalence of MOH in meconium but not UC.

For the benzodiazepine drug class, 11 analytes were considered, which included 8 drugs and select metabolites (Table I). UC positivity rates ranged between 0.3% and 1.0% compared to 0.1–0.6% for meconium, indicating higher positivity rates in UC. Oxazepam was the least frequently detected benzodiazepine in UC (0.3%) compared to lorazepam (0.1%)

Table I. Individual Drug Positivity Rates in Umbilical Cord and Meconium Utilizing Dataset-1 and Dataset-2

	Umbilical cord		Meconium		
	Positivity rate (%)	*N*	Positivity rate (%)	*N*	*P* value
Opioids					
6-Acetylmorphine	1.0%	39	0.0%	0	***
Codeine	3.7	149	2.2	90	***
Hydrocodone	3.5	142	2.1	84	***
Hydromorphone	5.0	201	2.5	102	***
Morphine	14.2	574	6.6	268	***
Oxycodone	2.4	97	1.8	73	ns
Oxymorphone	4.8	195	1.5	60	***
Methadone or EDDP	6.7	277	2.8	114	***
Buprenorphine or norbuprenorphine	15.6	630	9.1	367	***
Cannabinoids					
THC-COOH	25.3	535	35.0	739	***
Stimulants					
Amphetamine	6.7	269	7.7	311	ns
Methamphetamine	6.8	273	7.8	313	ns
Cocaine, benzoylecgonine, m-hydroxybenzoylecgonine or cocaethylene	4.0	162	5.6	227	***
Benzodiazepines					
Alprazolam or α-hydroxyalprazolam	1.0	39	0.3	10	***
Clonazepam or 7-aminoclonazepam	1.0	39	0.5	18	***
Diazepam	0.6	24	0.2	6	***
Nordiazepam	0.9	37	0.4	14	***
Lorazepam	0.4	16	0.1	4	**
Midazolam or α-hydroxymidazolam	0.6	24	0.6	24	ns
Oxazepam	0.3	12	0.2	8	ns
Temazepam	0.6	22	0.3	13	ns
Barbiturates					
Butalbital	1.9	77	1.0	40	***
Phenobarbital	0.1	5	0.2	8	*
Hallucinogens					
Phencyclidine	0.1	4	0.1	4	ns

*$P < 0.05$,
**$P < 0.01$,
***$P < 0.001$, ns (not significant).

in meconium. The most frequently detected benzodiazepines in UC were alprazolam and clonazepam (1.0%) in comparison to midazolam (0.6%) in meconium.

In the barbiturate drug class, the positivity rate for butalbital was twice as high in UC (1.91%) relative to meconium (1.0%, Table I). The phenobarbital positivity rate was slightly higher in meconium (0.2%) compared to UC (0.1%). In the hallucinogens drug class, phencyclidine was analyzed and had the same positivity rate (0.1%) for both specimen types.

Overall, for 32 analytes compared, the average analyte positivity rate was higher in UC (4.6%) compared to meconium (3.8%), by the approaches to testing available to us at the time of the study.

Umbilical cord and meconium had different multidrug patterns detected

Exposure to multiple drugs during pregnancy can occur (3, 4). To this end, we assessed the multidrug positivity status of the neonates by determining two- to three-drug combinations. Four- and five-drug combinations were associated with sample sizes of <5, and therefore, these combinations were not evaluated further. The most common two-drug combination detected in both specimen types was THC-COOH-opioids with a frequency of 7.1% in UC and 4.3% in meconium (Table II). The second, third and fourth common two-drug combinations between the two specimen types differed (Table II). The most common three-drug combination in both specimen types was opioid-amphetamine-cocaine (Table II).

Meconium had higher concentrations of opioids and THC-COOH

Studies comparing drug concentration in the two specimen types are lacking. We first investigated analyte concentrations in samples positive for opioid: codeine, 6-acetylmorphine, morphine, hydrocodone, hydromorphone, oxycodone, oxymorphone, methadone/2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and buprenorphine/norbuprenorphine. Opioid concentrations in meconium were overall higher than in UC (Figure 2A–C). Morphine had the highest concentration of all indicated opioids in both specimen types (Figure 2A). The median concentrations of morphine in meconium were 202.5 ng/g compared to 8.3 ng/g in UC, a 24.4-fold difference. Median codeine concentrations were 24.3-fold higher in meconium at 113.5 ng/g compared to 4.7 ng/g in UC. Hydrocodone concentrations were lower in meconium (89.5 ng/g) compared to codeine and morphine, yet 21.6-fold higher relative to that in UC (4.1 ng/g). Hydromorphone concentrations were the lowest of all indicated

Table II. Multidrug Positivity Rates in the Umbilical Cord and Meconium Specimens Utilizing Dataset-3

Umbilical cord			Meconium		
	Positivity rate (%)	*N*		Positivity rate (%)	*N*
Two-drug combination			Two-drug combination		
THC-COOH and opioids	7.5	151	THC-COOH and opioids	4.3	88
THC-COOH and morphine	3.7	75	THC-COOH and morphine	2.0	41
THC-COOH and norbuprenorphine	1.9	40	THC-COOH and norbuprenorphine	1.2	24
THC-COOH and EDDP	1.4	28	THC-COOH and hydrocodone	0.8	17
Opioids and amphetamines	1.7	35	THC-COOH and amphetamines	2.1	42
THC-COOH and amphetamines	1.0	21	Opioids and amphetamines	1.7	34
Opioids and cocaine	0.7	14	THC-COOH and cocaine	1.0	21
Three-drug combination			Three-drug combination		
Opioids, amphetamines and cocaine	0.6	12	Opioids, amphetamines and cocaine	0.5	11

opioids (22.8 ng/g) in meconium but 8.14-fold higher than in UC (2.8 ng/g). Oxycodone and oxymorphone concentrations in meconium were 104.0 and 71.0 ng/g, respectively, relative to those in UC at 3.0 and 2.5 ng/g, suggesting an average of 31.3-fold higher concentration in meconium. Methadone had the highest of all opioid concentrations in both specimen types (Figure 2B). The median methadone concentration in meconium was 4,616 ng/g, while that in UC was 67.68 ng/g, a 68.2-fold difference. Similarly, the methadone metabolite EDDP was detected at a median concentration of 5,000 ng/g in meconium compared to 2.7 ng/g in UC. It is noteworthy that the concentrations of methadone and EDDP were near equivalent in meconium, but the parent drug was the primary analyte detected in UC. Buprenorphine and norbuprenorphine were detected at 96.0 and 395.3 ng/g, respectively, in meconium as opposed to 1.8 and 4.3 ng/g, respectively, in UC, suggesting an average of 74.5-fold difference between the two specimen types (Figure 2C).

Next, we compared THC-COOH and phencyclidine concentrations in the two specimen types. The median THC-COOH concentration in meconium was 36-fold higher at 72.0 ng/g when compared to 2.0 ng/g in UC (Figure 3A). Collectively, for opioids and THC-COOH, meconium had an average of 37.9 times higher drug concentration than UC.

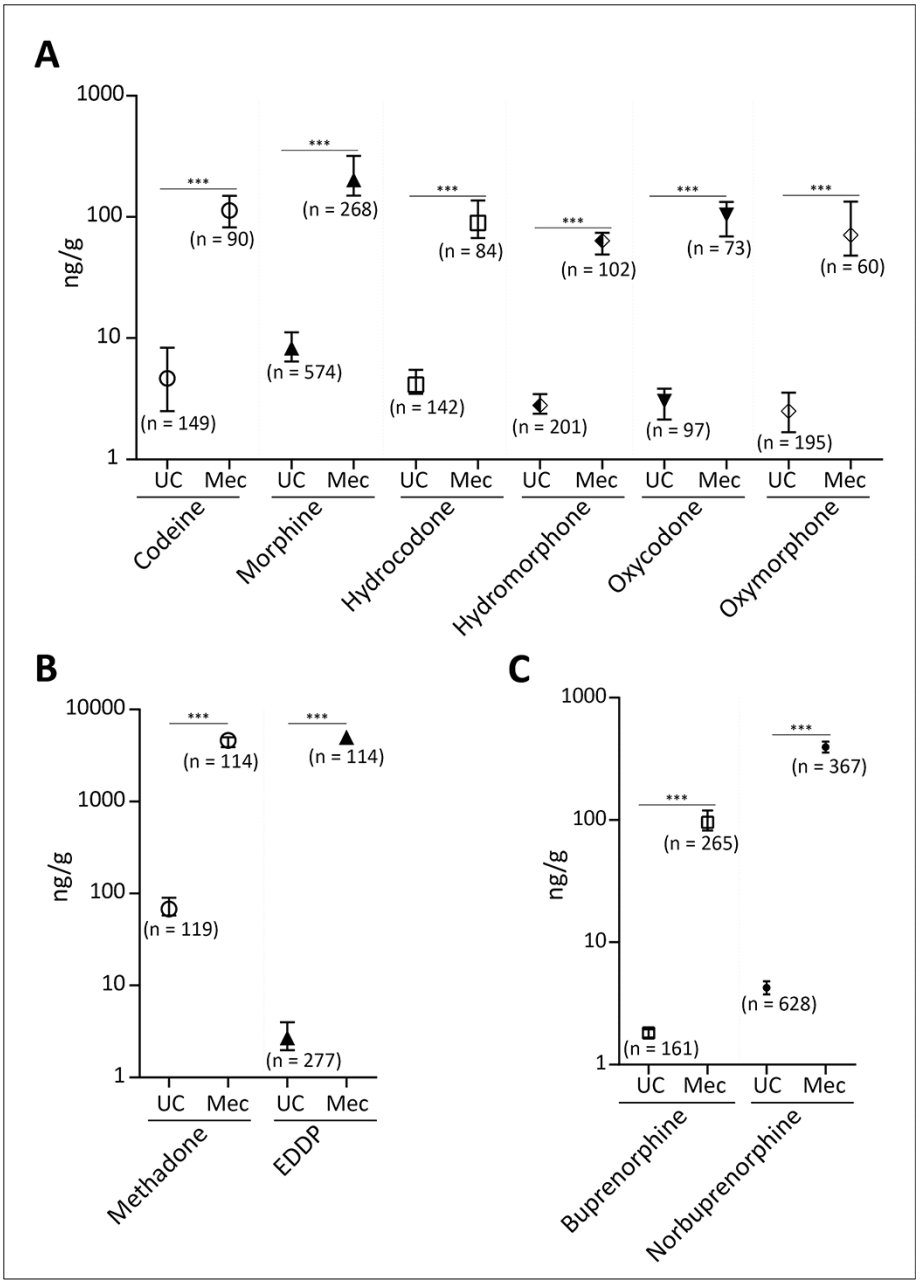

Figure 2. Comparison of opioids concentrations in UC and meconium specimens using Dataset-1 and Dataset-2. (A) Median concentrations and 95% CI of indicated opioid analytes in UC and meconium specimens. (B) Median concentrations and 95% CI of methadone and metabolite EDDP in UC and meconium specimens. (C) Median concentrations and 95% CI of buprenorphine and metabolite norbuprenorphine in UC and meconium specimens. The Wilcoxon matched-pairs signed-rank test was performed to calculate statistical significance between groups. ***$P < 0.001$. Numbers in parentheses indicate the number of positive specimens in that group.Abbreviation: CI: confidence interval.

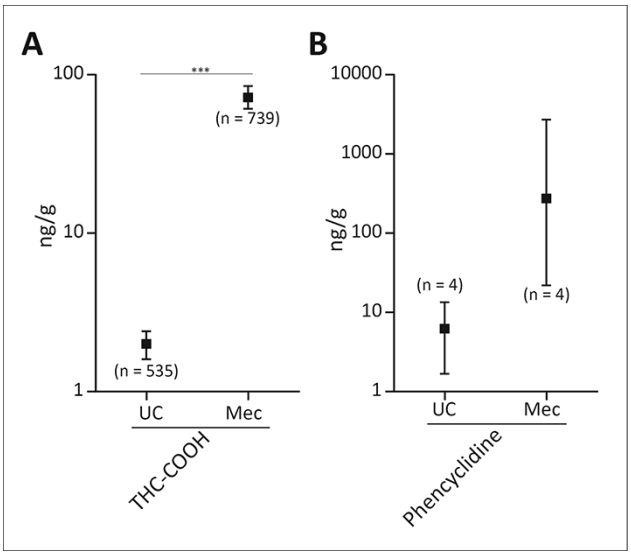

Figure 3. Comparison of 11-Nor-9-carboxy-THC and phencyclidine concentrations in UC and meconium specimens using Dataset-2 and Dataset-1. (A) Median concentrations and 95% CI of THC-COOH in UC and meconium specimens. (B) Median concentrations and 95% CI of phencyclidine in UC and meconium specimens. ***$P < 0.001$. Numbers in parentheses indicate the number of positive specimens in that group.Abbreviation: CI: confidence interval.

Lastly, we investigated drug concentrations for phencyclidine, which showed a median concentration of 275.0 ng/g in meconium compared to 6.2 ng/g in UC, although there was no statistically significant difference between the two specimen types, likely due to the small sample size (Figure 3B).

Amphetamine and cocaine analytes were detected at higher concentrations in meconium

In the stimulant drug class, we investigated median concentrations of amphetamine, methamphetamine as well as cocaine and metabolites (i.e., benzoylecgonine, MOH and cocaethylene). The overall stimulant concentrations were higher in meconium than in UC (Figure 4A and B). Methamphetamine had the highest of all stimulant analyte concentrations, while cocaethylene had the lowest concentration (Figure 4A). Median amphetamine concentrations in meconium were 7.2-fold higher at 364.0 ng/g relative to those in UC at 50.6 ng/g. Methamphetamine concentrations were much higher in both specimen types with 1,000 ng/g in meconium compared to 147.6 ng/g in UC. The ratio of methamphetamine to amphetamine was similar for both specimen types. For cocaine and metabolites, benzoylecgonine had the highest concentrations in both specimen types, while cocaethylene had the lowest (Figure 4B). Median cocaine concentration in meconium was 78.8-fold higher at 316.0 ng/g compared to 4.01 ng/g in UC. While benzoylecgonine concentration in meconium was higher (519.0 ng/g) than in cocaine, the fold difference between meconium and UC concentrations was much smaller (15.0) due to higher concentrations (34.5 ng/g) observed in UC. Benzoylecgonine was also the most commonly detected cocaine analyte in UC. The metabolite MOH, which is more frequently detected in meconium had a 20.9-fold higher concentration in meconium with a median concentration of 144.7 ng/g compared to 6.9 ng/g. Lastly, cocaethylene, which is formed due to simultaneous consumption of cocaine and ethanol, had a 59.6-fold higher concentrations in meconium at a median concentration of 105.5 ng/g compared to 1.8 ng/g in UC. Thus, amphetamines and cocaine drug analytes showed an average of 36.3-fold higher concentrations in meconium relative to UC.

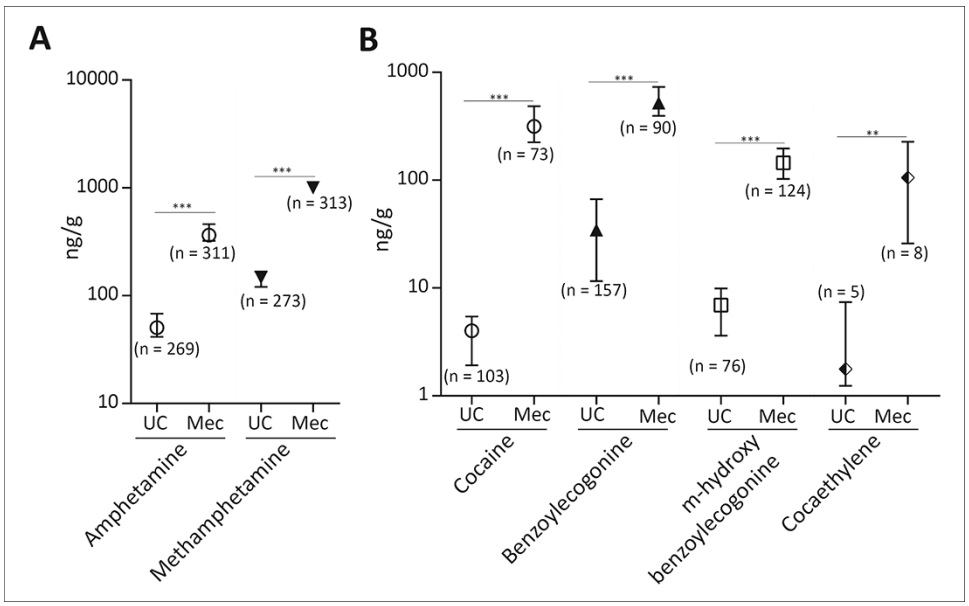

Figure 4. Comparison of stimulant drugs and their metabolite concentrations in UC and meconium specimens utilizing Dataset-1. (A) Median concentrations and 95% CI of amphetamine and methamphetamine in UC and meconium specimens. The Wilcoxon matched-pairs signed-rank test was performed to calculate statistical significance between groups. (B) Median concentrations and 95% CI of cocaine and indicated metabolites in UC and meconium specimens. Wilcoxon matched-pairs signed-rank test was performed to calculate statistical significance between groups. **$P < 0.01$, ***$P < 0.001$. Numbers in parentheses indicate the number of positive specimens in that group.Abbreviation: CI: confidence interval.

Benzodiazepines and barbiturates concentrations were higher in meconium

In the benzodiazepines drug class, we investigated eight drugs including alprazolam, clonazepam, lorazepam, diazepam, nordiazepam, oxazepam, midazolam and temazepam and their metabolites when available for analysis. The overall concentrations for all benzodiazepines in meconium were greater than that in UC (Figure 5A and B). Median benzodiazepine concentrations in meconium ranged between 33.0 and 93.0 ng/g, wherein midazolam had the highest and clonazepam had the lowest concentrations in meconium (Figure 5A and B). Of note, the concentrations of clonazepam and 7-aminoclonazepam were near equivalent in UC and were found together in nearly all specimens, but in meconium, the parent drug concentration was higher and was detected in roughly a third of the meconium samples (Figure 5A). The overall median benzodiazepine concentrations in UC ranged between 1.9 and 12.5 ng/g, wherein α-hydroxyalprazolam had the lowest, while nordiazepam had the highest concentrations. Thus, on average, meconium had an 8.5-fold higher concentration of benzodiazepines compared to UC.

In the barbiturate drug class, we analyzed butalbital and phenobarbital. While butalbital was slightly more concentrated in meconium (401.0 ng/g) than in UC (341.9 ng/g), the distribution was comparable (Figure 5C). In contrast, phenobarbital, which is sometimes administered directly to a newborn after birth, was significantly more concentrated in meconium (5,000 ng/g) compared to UC (151.0 ng/g)—a 33.1-fold difference.

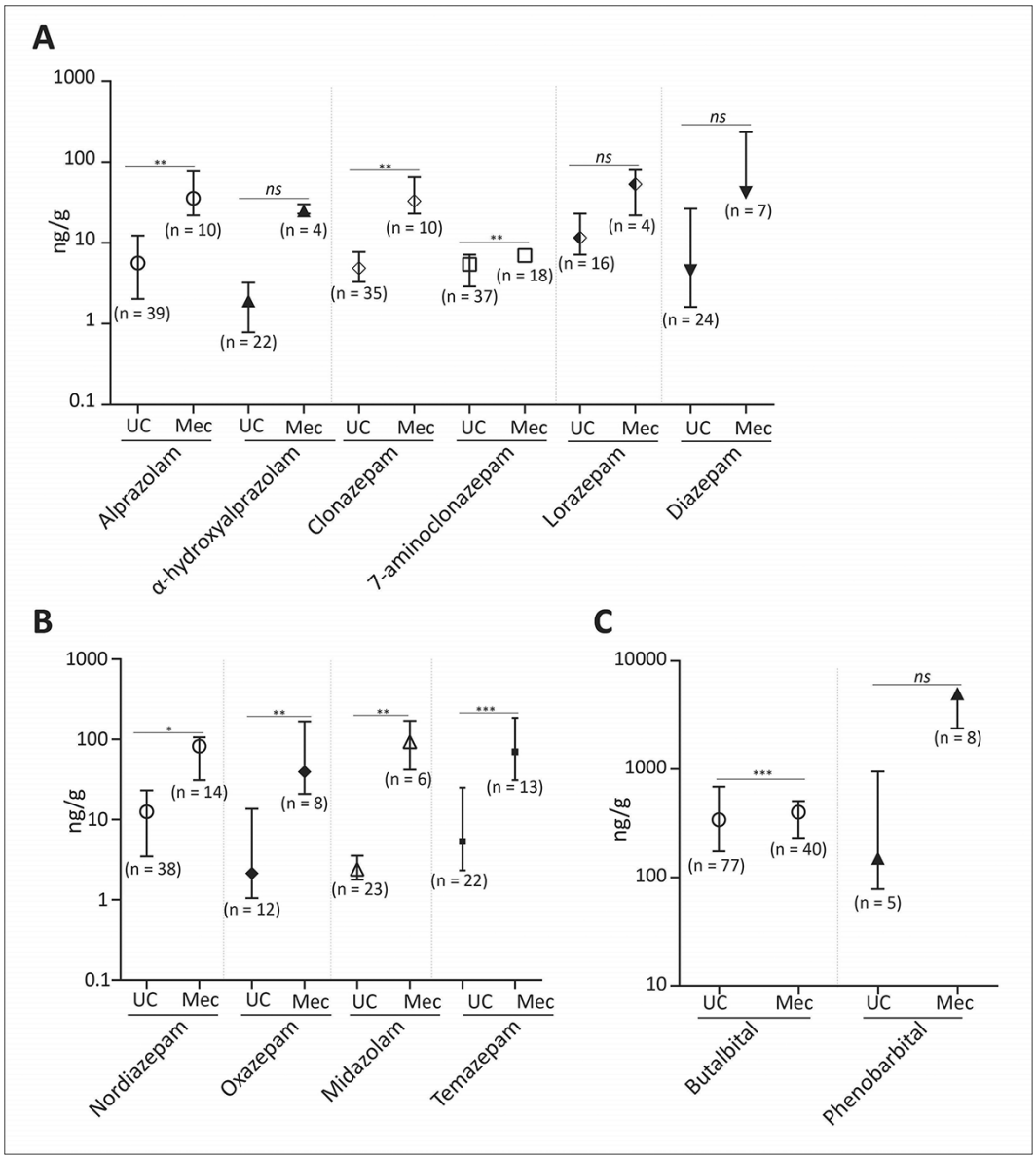

Figure 5. Comparison of benzodiazepines, barbiturates and their metabolite concentrations in UC and meconium specimens utilizing Dataset-1. (A) Median concentrations and 95% CI of indicated benzodiazepine analytes in UC and meconium specimens. (B) Median concentrations and 95% CI of indicated benzodiazepine analytes in UC and meconium specimens. (C) Median concentrations and 95% CI of indicated barbiturates in UC and meconium specimens. The Wilcoxon matched-pairs signed-rank test was performed to calculate statistical significance between groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Numbers in parentheses indicate the number of positive specimens in that group.Abbreviation: CI: confidence interval.

Discussion

This study compared drug concentrations in 4,036 paired UC and meconium specimens. The drug concentrations were overall higher in meconium, apart from 7-aminoclonazepam and phencyclidine. These higher concentrations may be attributed to several factors. Most compounds included in our drug panels are relatively hydrophobic (4). Since meconium is significantly more hydrophobic than Wharton's jelly in UC where drug compounds are deposited (3, 19, 20), analyte accumulation may be favored in meconium. In addition, meconium is produced based on the elimination of constituents delivered to the fetal circulation through the placenta and by repeated ingestion of amniotic fluid by the fetus (19, 20), which in turn may lead to higher analyte concentrations compared to UC, wherein the drugs may pass through the UC vasculature and Wharton's jelly only once. Also, UC tissue is bathed in the amniotic fluid in which cells may be sloughed as the UC grows during pregnancy. As such, drug analytes could theoretically be shed into the amniotic fluid and subsequently incorporated into the meconium, which is less likely to be sloughed within the fetus.

The specific mechanism of drug deposition and retention in UC is not well characterized. In addition, meconium is believed to accumulate from the time it begins to form until it is passed after birth, whereas the composition of the UC is likely to change over time until collected at birth. The efficiency of placental transfer of the individual compounds may also impact drug concentrations in the two specimen types (3, 20). Hydrophobic analytes are more likely to be excreted in bile, and given that meconium is also hydrophobic, it can be speculated that these compounds may be more enriched in meconium than UC. In addition, the duration, extent and timing of maternal drug exposure can also determine drug concentrations in the two matrices. For instance, drugs when given during labor and delivery (e.g. morphine) are more likely to be detected in UC, which often contains residual maternal blood (3, 20). In contrast, drugs administered to the infant immediately after birth (e.g. phenobarbital) will not be detected in UC but may be detected in meconium if the drug(s) is incorporated in the meconium prior to specimen collection.

In vivo stability of compounds may also affect their concentrations and detection. For instance, the *in vivo* plasma half-life of 6-acetylmorphine is only 6–25 min before being hydrolyzed to morphine (21). Therefore, it is likely that only very recent heroin use can be definitively detected in meconium or UC, and it is more likely that 6-acetylmorphine will be detected in UC if the assay cut-off is sufficiently low. Finally, the quality of the specimens submitted for testing may also impact drug concentrations. For instance, meconium contaminated with milk stool may reduce the detected drug concentrations through dilution of the meconium (22, 23). Very small UC specimens are also likely to contain little Wharton's jelly and thereby reduce the opportunity for drug deposition. As well, proper labeling, storage and transport of specimens are additional factors affecting the stability and detection of compounds. Thus, perinatal/postnatal drug exposure and the physico-chemical properties of both matrices, chemical properties of the drug analytes, nature of maternal drug exposure, fetal physiology and preanalytical factors may collectively differentiate drug concentration in UC versus meconium.

Detection of drugs in UC and meconium depends on the factors described above but also on the analytical approach to testing. We compared drug positivity rates between UC and meconium specimen types and observed overall higher positivity rates in the former. Many analytical variables contribute to this observation. The assay design at our institution for meconium testing includes an immunoassay-based initial test (drug class-based screens) followed by confirmatory testing of samples that test positive in the initial test, using LC–MS-MS assays (Supplementary Table S1). In contrast, UC specimens are tested using a targeted large multi-class LC–MS-MS panel for all drugs except THC-COOH, which is detected separately by LC–MS-MS. Importantly, the positivity cut-offs used for the immunoassay screen are typically higher than the confirmatory cut-offs for meconium testing (Supplementary Table S1), and cross-reactivity for individual compounds varies based on the capture antibody included in the assay design. As a result, meconium specimens with drug cross-reactivity that falls below that of the assay calibrator (prepared at the assay cut-off concentration) do not get reflexed to the confirmatory assay.

Cross-reactivity in class-based immunoassays may be highly variable for individual drug analytes, whereas sensitivity for individual drug analytes is known in a targeted LC–MS-MS assay (24, 25). For instance, the meconium assay for opiates uses morphine as a calibrator. Cross-reactivity for other opioids in this assay is highly variable and sometimes sufficiently poor to produce false-negative results. In contrast, the UC assay specifically targets hydrocodone, oxycodone and oxymorphone as well as metabolites (i.e. norhydrocodone, noroxycodone and noroxymorphone). Furthermore, because drug concentrations in UC are lower than in meconium, the positivity cut-offs of the LC–MS-MS assays for UC testing were designed to be on average 4.13 times lower than the meconium assays. The meaningfulness and appropriateness of the cut-off concentrations are not known. For example, studies designed to evaluate the clinical sensitivity of UC and meconium are limited, so there is no standardization of cut-off concentrations in these specimen types (26). It is known that meconium and UC testing may not identify drugs despite a newborn exhibiting signs and symptoms of withdrawal (20, 27). Thus, a high sensitivity targeted assay design used for UC combined with lack of a high cut-off immunoassay screen contributes to making the UC testing in our institution analytically more sensitive than meconium testing, but doing so may not have improved clinical sensitivity of newborn drug testing.

Meconium has been considered the gold standard to evaluate drug detection status in newborns (28). However, both specimen types are utilized for this purpose, and there is an ongoing debate on the suitability of one over the other (6–8). Several points need to be considered to make that determination. Individual hospital logistics and practices such as universal collection, collection for cause, staffing and involvement of the laboratory play important roles. Overall, it might be easier to collect UC due to the immediate availability of specimen and presence of medical staff at the time of birth. However, specialized training and planning may be required to ensure proper cleaning, labeling and transport of UC specimens as improper collection and transport may compromise specimen quality and the overall diagnostic yield of drug testing. Regarding meconium, several factors such as

Table III. Comparative Summary of UC and Meconium Specimens in Assessing Neonatal Drug Exposure

Umbilical cord	Meconium
Drug compound accumulation is more dynamic since UC is a live tissue	Drug compounds accumulate over time
Specimen collection at birth is relatively straightforward but may require special training in cleaning and transport	Specimen collection can be challenging due to the unpredictable nature of infants passing their first stool
Small UC specimens may reduce the opportunity for drug detection	Intrauterine meconium release or breast milk contamination may reduce the opportunity for drug detection
Likely to yield faster turnaround times	Likely to yield slower turnaround times
Overall drug positivity rates are higher by current methodologies	Overall drug concentration is higher

the intrauterine release of meconium, collection of multiple passages after birth that might be mixed with digested breast milk as well as intentional discarding of specimens by parents due to the fear of consequences may hinder proper specimen collection.

In this study, we showed side-by-side comparisons of drug concentrations, positivity rates and the detection of multidrug combinations. It is important to highlight some of the limitations as well. For instance, only those analytes available for both specimen panels were compared in this study. Also, as previously mentioned, drug positivity rates in meconium are dependent on the immunoassay results, which have higher cut-off values than confirmatory testing, and may underestimate the meconium positivity rates. Also, it is important to mention that this study compares the performance of different assays, which may differ for reasons other than the matrix. Furthermore, the drug positivity rates described here only represent a subset of clients that submitted paired UC and meconium specimens for routine clinical testing. These clients are located in 13 US states. Hence, the possibility that specimens submitted from other parts of the country may show different results cannot be excluded. Due in large part to geographical differences in demand for detection of marijuana exposure, the THC-COOH testing in UC must be requested at our institution separately from the drug panel, whereas THC-COOH testing is part of the meconium drug panel. The high positivity rate observed for THC-COOH could be attributed to marijuana legalization in the USA. In most states of the country, medicinal and/or recreational marijuana has been legalized or decriminalized (29). Furthermore, exposure to cannabidiol products that contain trace amounts of THC-COOH may also lead to the presence of this analyte in fetal specimens. Therefore, a possibility of bias in UC THC-COOH results cannot be excluded. Lastly, it is imperative to highlight that the number of data points in the UC and meconium data sets differs. As a result, inclusion of low concentration datapoints in one data set (i.e., UC) but not the other may skew the median.

Despite these limitations, this study is the largest study ($n = 4{,}036$) conducted on paired UC and meconium specimens that we are aware of, which provides high statistical power. Furthermore, it allows a side-by-side comparison of the drug detection status in UC versus meconium specimens as opposed to separate investigations in each specimen type. Also, multidrug positivity patterns detected in each specimen type are reported here, which may provide crucial insight into the common patterns of multidrug use in pregnant women as well as the likely multidrug patterns that might be observed if testing meconium and/or UC. It is important to mention, however, that the multidrug patterns may not necessarily be interpreted as multidrug exposures unless the detected compounds belong to completely different drug classes. Moreover, the detection of different multidrug patterns in UC and meconium specimens may confound the interpretation of multidrug positivity with multidrug exposure by clinical providers. Lastly, the results of this study suggest that a more sensitive and specific assay design may improve drug detection rates in meconium.

In conclusion, utilizing a large retrospective cohort of paired meconium and UC specimens, we showed that meconium has a higher concentration of drugs. Despite this, drug positivity rates are higher in UC, due largely to differences in analytical approach at our institution, including much lower cut-off concentrations for UC than for meconium. Furthermore, the detection of different multidrug combinations detected in the two matrices underscores the fact that maternal factors, specimen characteristics and assay design should be considered when making a choice for specimen type for evaluating neonatal drug exposure (Table III).

Supplementary Data

Supplementary data are available at *Journal of Analytical Toxicology* online.

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List of abbreviations

UC Umbilical cord tissue
LC–MS-MS Liquid chromatography-tandem mass spectrometry
ELISA Enzyme-linked immunosorbent assay
THC THC-COOH (–)-trans-Δ9-tetrahydrocannabinol, the primary psychoactive component of marijuana 11-Nor-9-carboxy-THC
MOH m-Hydroxybenzoylecgonine
EDDP 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
TAT Turnaround time

References

1. Rentsch, K.M. (2020) Drug exposure in newborns: effect of selected drugs prescribed to mothers during pregnancy and lactation. *Therapeutic Drug Monitoring*, **42**, 255–263.
2. Behnke, M., Smith, V.C. (2013) Prenatal substance abuse: short- and long-term effects on the exposed fetus. *Pediatrics*, **131**, e1009–e1024.
3. Wabuyele, S.L., Colby, J.M., McMillin, G.A. (2018) Detection of drug-exposed newborns. *Therapeutic Drug Monitoring*, **40**, 166–185.
4. Ross, E.J., Graham, D.L., Money, K.M., Stanwood, G.D. (2015) Developmental consequences of fetal exposure to drugs: what we know and what we still must learn. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, **40**, 61–87.
5. Miranda, L., Dixon, V., Reyes, C. (2015) *How States Handle Drug Use During Pregnancy*. *ProPublica* https://projects.propublica.org/graphics/maternity-drug-policies-by-state (accessed May 28, 2021).
6. Colby, J.M., Adams, B.C., Morad, A., Presley, L.D., Patrick, S.W. (2019) Umbilical cord tissue and meconium may not be equivalent for confirming in utero substance exposure. *The Journal of Pediatrics*, **205**, 277–280.
7. Labardee, R.M., Swartzwelder, J.R., Gebhardt, K.E., Pardi, J.A., Dawsey, A.C., Brent Dixon, R., et al. (2017) Method performance and clinical workflow outcomes associated with meconium and umbilical cord toxicology testing. *Clinical Biochemistry*, **50**, 1093–1097.
8. Colby, J.M. (2017) Comparison of umbilical cord tissue and meconium for the confirmation of in utero drug exposure. *Clinical Biochemistry*, **50**, 784–790.
9. Black, E., Khor, K.E., Kennedy, D., Chutatape, A., Sharma, S., Vancaillie, T., et al. (2019) Medication use and pain management in pregnancy: a critical review. *Pain Practice*, **19**, 875–899.
10. McMillin, G.A., Wood, K.E., Strathmann, F.G., Krasowski, M.D. (2015) Patterns of drugs and drug metabolites observed in meconium: what do they mean? *Therapeutic Drug Monitoring*, **37**, 568–580.
11. ElSohly, M.A., Stanford, D.F., Murphy, T.P., Lester, B.M., Wright, L.L., Smeriglio, V.L., et al. (1999) Immunoassay and GC-MS procedures for the analysis of drugs of abuse in meconium. *Journal of Analytical Toxicology*, **23**, 436–445.
12. Marin, S.J., Merrell, M., McMillin, G.A. (2011) Drugs of abuse detection in meconium: a comparison between ELISA and biochip microarray. *Journal of Analytical Toxicology*, **35**, 40–45.
13. Chittamma, A., Marin, S.J., Williams, J.A., Clark, C., McMillin, G.A. (2013) Detection of in utero marijuana exposure by GC-MS, ultra-sensitive ELISA and LC-TOF-MS using umbilical cord tissue. *Journal of Analytical Toxicology*, **37**, 391–394.
14. Marin, S.J., Metcalf, A., Krasowski, M.D., Linert, B.S., Clark, C.J., Strathmann, F.G., et al. (2014) Detection of neonatal drug exposure using umbilical cord tissue and liquid chromatography time-of-flight mass spectrometry. *Therapeutic Drug Monitoring*, **36**, 119–124.
15. Okoye, N.C., McMillin, G.A. (2021) Patterns of neonatal co-exposure to gabapentin and commonly abused drugs observed in umbilical cord tissue. *Journal of Analytical Toxicology*, **45**, 506–512.
16. Wu, F., Jensen, T.L., McMillin, G.A. (2019) Detection of in utero cannabis exposure in umbilical cord tissue by a sensitive liquid chromatography-tandem mass spectrometry method. *Methods in Molecular Biology (Clifton, N.J.)*, **1872**, 211–222.
17. Jensen, T.L., Wu, F., McMillin, G.A. (2019) Detection of in utero exposure to cannabis in paired umbilical cord tissue and meconium by liquid chromatography-tandem mass spectrometry. *Clinical Mass Spectrometry*, **14**, 115–123.
18. Nelson, H.A., Wood, K.E., McMillin, G.A., Krasowski, M.D. (2021) Umbilical cord drug screening in multiple births: experience from a reference laboratory and academic medical center. *Journal of Analytical Toxicology*, 1–7.
19. Palmer, K.L., Krasowski, M.D. (2019) Alternate matrices: meconium, cord tissue, hair, and oral fluid. *Methods in Molecular Biology (Clifton, N.J.)*, **1872**, 191–197.
20. Farst, K.J., Valentine, J.L., Hall, R.W. (2011) Drug testing for newborn exposure to illicit substances in pregnancy: pitfalls and pearls. *International Journal of Pediatrics*, **2011**, 951616.
21. Ellis, A.D., McGwin, G., Davis, G.G., Dye, D.W. (2016) Identifying cases of heroin toxicity where 6-acetylmorphine (6-AM) is not detected by toxicological analyses. *Forensic Science, Medicine, and Pathology*, **12**, 243–247.
22. Gray, T.R., LaGasse, L.L., Smith, L.M., Derauf, C., Grant, P., Shah, R., et al. (2009) Identification of prenatal amphetamines exposure by maternal interview and meconium toxicology in the Infant Development, Environment and Lifestyle (IDEAL) study. *Therapeutic Drug Monitoring*, **31**, 769–775.
23. Wabuyele, S.L., Colby, J.M., McMillin, G.A. (2018) Detection of drug-exposed newborns. *Therapeutic Drug Monitoring*, **40**, 166–185.
24. Swortwood, M.J., Hearn, W.L., DeCaprio, A.P. (2014) Cross-reactivity of designer drugs, including cathinone derivatives, in commercial enzyme-linked immunosorbent assays. *Drug Testing and Analysis*, **6**, 716–727.
25. Reschly-Krasowski, J.M., Krasowski, M.D. (2018) A difficult challenge for the clinical laboratory: accessing and interpreting manufacturer cross-reactivity data for immunoassays used in urine drug testing. *Academic Pathology*, **5**, 2374289518811797.
26. Wood, K.E., Sinclair, L.L., Rysgaard, C.D., Strathmann, F.G., McMillin, G.A., Krasowski, M.D. (2014) Retrospective analysis of the diagnostic yield of newborn drug testing. *BMC Pregnancy and Childbirth*, **14**, 250.
27. Moore, C., Lewis, D., Leikin, J. (1995) False-positive and false-negative rates in meconium drug testing. *Clinical Chemistry*, **41**, 1614–1616.
28. Price, H.R., Collier, A.C., Wright, T.E. (2018) Screening pregnant women and their neonates for illicit drug use: consideration of the integrated technical, medical, ethical, legal, and social issues. *Frontiers in Pharmacology*, **9**, 961.
29. Marijuana Policy Project. (2021) *2021 Marijuana Policy Reform Legislation*. https://www.mpp.org/issues/legislation/key-marijuana-policy-reform/ (accessed June 15, 2021).